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Research Note—

Cecal Colonization of Chicks by Bovine-Derived Strains of Campylobacter

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SUMMARY. *Campylobacter jejuni* and *Campylobacter coli* strains were isolated from feces of dairy cattle at farms with no known problem due to campylobacteria. Farms were located in the northeast, desert southwest, and Pacific west. Twenty isolates were identified by ribotyping with a RiboPrinter.® The ability of these bovine isolates to colonize the ceca of chicks was determined by challenge inoculation and reisolation of the challenge strain from the ceca at 1 and 2 wk after challenge. Isolates recovered from chick ceca were examined by ribotyping to assure they matched the challenge strain. One hundred percent of the bovine-derived challenge strains were capable of colonizing chicks. These results indicate that dairy cattle may be asymptomatic *Campylobacter* carriers and potential sources of campylobacteria contamination of poultry facilities.

RESUMEN. *Nota de Invesigación*—Colonización cecal de los pollitos con cepas de Campylobacter de origen bovino.

Se aislaron cepas de *Campylobacter jejuni* y *Campylobacter coli* a partir de heces de ganado lechero en granjas donde no existían problemas de Campylobacteriosis. Las granjas estaban localizadas en las regiones del nordeste, sureste y Pacífica de Estados Unidos. Se identificaron 20 aislados mediante la ribotipificación con un RiboPrinter.® La capacidad de estos aislados de origen bovino para colonizar el ciego fue determinada mediante la inoculación y el aislamiento de la cepa de desafío a partir del ciego de pollitos 1 y 2 semanas después del desafío. Los aislados obtenidos de los ciegos de pollitos fueron examinados mediante la ribotipificación con el objeto de compararlos con las cepas inoculadas. El 100% de los aislados de origen bovino colonizó los pollitos. Estos resultados indican que el ganado lechero puede ser portador asintomático de *Campylobacter* y pueden ser fuentes potenciales de contaminación para las instalaciones avícolas.

Key words: *Campylobacter*, dairy cattle, bovine, colonization, chick, poultry, ceca

Abbreviation: CFU = colony-forming units

Poultry are a major source of *Campylobacter* infections in humans, but how the organism gains access to broiler facilities is unclear. Theories include spread from animal reservoirs (5), presence of *Campylobacter* in water (18), presence of viable but nonculturable forms in water (17), spread by rodent and insect vectors (5), contamination in hatcheries (7), and vertical transmission through the breeder stock (2,3). It is possible that each of these plays some role.

Gregory *et al.* (5) suggested that cattle may be a reservoir maintaining the presence of *Campylobacter* on the farm. However, they presented no molecular epidemiologic evidence demonstrating that strains present in cattle were clonal with those present in the poultry house. Evidence that cattle are sources of poultry isolates is mostly circumstantial and mixed.

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Stanley and Jones (15) studied resistance to the antibiotic metronidazole in 2157 *C. jejuni* strains. Poultry isolates tended to be resistant (90% of the broiler isolates and 92% of the turkey isolates), whereas only 19% of isolates from dairy cattle were resistant to metronidazole. Aarestrup *et al.* (1) studied resistance to 16 antimicrobials among *Campylobacter* isolates from humans, pigs, and cattle. Differences in resistance patterns were noted according to the source species. Some authors have used serotyping schemes to assess the relatedness of isolates from different species (1,8,9,11), and recently a variety of more reliable and accurate molecular methods have become available for epidemiologic investigations (10,12, 13). For example, Owen and Leeton (13) studied restriction fragment length polymorphism within the flagellin gene and found that *Campylobacter* strains with the same *flaA* type were recoverable from different hosts.

Several years ago it was reported that *Campylobacter* isolates from chickens were sometimes incapable of colonizing other chickens (16). Additionally, *Campylobacter* strains vary in their colonizing ability (4,16,20). The reports by Stas *et al.* (16) and Glunder (4) raised some issues concerning host range and adaptability of various strains of campylobacteria. Because *Campylobacter* isolates from poultry are not always capable colonizers of other chickens, a question arises as to whether or not host range specificities limit the movement of strains among animal species. For example, are bovine strains somewhat host adapted and therefore tend not to spread to poultry? Such a phenomenon seems to be indicated by the distribution of antibiotic resistance among isolates from different species (1,15). To our knowledge, no study has directly demonstrated the ability or inability of *Campylobacter* strains obtained from cattle to colonize the chicken. In the current study, we evaluated the poultry colonizing ability of *Campylobacter* isolates obtained from the feces of lactating dairy cows at farms in widely divergent geographic areas of the United States: the northeast (New York), desert southwest (Arizona and New Mexico), and Pacific west (California).

MATERIALS AND METHODS

Animals. Day-of-hatch leghorn chickens (HyLine W-36®) were obtained from a commercial hatchery (HyLine International, Bryan, TX) and placed in electrically heated commercial brooder batteries, 10 chicks per cage. Feed was heat sterilized in an oven at 65 C for 24 hr. Chlorinated municipal drinking water was provided in open troughs. Chicks were provided water and a balanced unmedicated corn-soybean ration *ad libitum.* An Institutional Animal Care and Use Committee reviewed and approved husbandry and experimental procedures.

Sources of *Campylobacter* strains. A separate study (6) was done to determine the prevalence of *Campylobacter* in lactating dairy cows. *Campylobacter* used in this present work were strains isolated from that work. Briefly, fecal samples were collected from 720 cows on farms with no prior history of *Campylobacter* problems. Samples were collected from four farms in the northeast (New York), four farms in the desert southwest (Arizona and New Mexico), and four farms in the Pacific west (California). *Campylobacter* were isolated, identified with the INDX®-Campy(jcl)™ latex agglutination test (Integrated Diagnostics, Baltimore, MD), and further characterized with a RiboPrinter® Microbial Characterization System (Qualicon, Wilmington, DE). Twenty isolates with distinctly different ribotypes were selected for use in the present study. Two isolates were *C. coli* strains; the rest were *C. jejuni*.

Experimental design. Colonization studies were conducted with each of the bovine-derived *Campylobacter* strains listed in Table 1. Each study included a brooder battery containing 10 untreated control chicks as indicators of *Campylobacter* contamination from either the hatchery or within our facility. Up to six additional brooder batteries containing 10 chicks each were placed in our isolation facility at the same time as the control group. *Campylobacter* strains were grown on campy-cefex agar (19) at 42 C for approximately 42 hr. The plates were washed with water, and the resultant suspension was serially diluted to attain a suspension containing approximately 10^7 colony-forming units (CFU)/ml. Day-of-hatch chicks were inoculated by gastric gavage with 1 ml of the cell suspension. We humanely killed five chicks from each group, including the control group, at 1 and 2 wk after challenge inoculation. Cecal contents were collected, serially diluted, and plated on campy-cefex agar (19). *Campylobacter* colony counts were obtained for cecal material collected from each chick. Well-isolated colonies were picked for ribotyping.

Ribotyping. We used a RiboPrinter® microbial characterization system to compare the strains used for inoculation with the strain recovered from inoculated chickens. The RiboPrinter® analyzes the 5, 16, and 23 S RNA regions of ribosomal RNA to characterize bacterial samples. It then automatically compares sample data with existing patterns within a database library and, thereby, can identify an unknown organism. Further, the system can also be used to determine if isolates from inoculated chickens are the same as the challenge strain. However, in some instances the built-in mathematical analysis generates results that require

Table 1. Colonization of chick ceca by various bovine-derived strains of *Campylobacter*.

Sample no.	Source[A]	Mean concentration[B] of *Campylobacter* in chicks at 1 wk after challenge	Mean concentration[B] of *Campylobacter* in chicks at 2 wk after challenge	Ribotyping confirmation[C]	
				Week 1	Week 2
1	New Mexico A	7.86 ± 1.03	7.73 ± 1.25	Yes	Yes
16	New York C	8.38 ± 0.83	8.74 ± 0.25	Yes	Yes
19	New York C	8.10 ± 0.19	8.19 ± 0.24	Yes	Yes
22	New Mexico B	5.25 ± 0.33	8.74 ± 0.22	Yes	Yes
31	New Mexico B	6.75 ± 0.78	8.65 ± 0.94	Yes	Yes
37	New Mexico B	2.44 ± 3.44	6.50 ± 3.75	Yes	Yes
40	New Mexico B	8.23 ± 0.44	8.20 ± 0.32	Yes	Yes
43	New Mexico B	7.85 ± 0.70	8.45 ± 0.36	Yes	Yes
46	New Mexico B	7.43 ± 0.81	6.11 ± 3.44	Yes	Yes
55	New York D	6.07 ± 3.45	7.98 ± 1.00	Yes	Yes
58[D]	California A	8.18 ± 0.46	8.47 ± 0.58	Yes	Yes
61	California A	7.84 ± 1.14	8.03 ± 0.56	Yes	Yes
64	California A	7.98 ± 0.67	8.87 ± 0.41	Yes	Yes
67[D]	California A	7.52 ± 0.47	8.70 ± 0.39	Yes	Yes
70	California A	8.23 ± 0.88	8.62 ± 0.49	Yes	Yes
73	California A	No growth	8.81 ± 0.99	NA[E]	Yes
76	New Mexico A	7.97 ± 0.35	8.70 ± 0.30	Yes	Yes
79	Arizona C	7.63 ± 0.26	9.08 ± 0.36	Yes	Yes
82	Arizona D	7.72 ± 0.44	8.31 ± 0.56	Yes	Yes
91	Arizona D	6.05 ± 3.45	8.66 ± 0.51	Yes	Yes

[A]Different farms within each state are designated by different capital letters.
[B]Mean and standard deviations of *Campylobacter* concentration per gram of cecal material expressed as $\log_{10}$ transformations of the plate counts, n = 5.
[C]*Campylobacter* strains recoverd from inoculated chicks matched the ribotype of the challenge strain.
[D]*Campylobacter coli* strains.
[E]NA = not applicable.

human review. In these cases, the printed banding patterns were visually compared with each other.

Data analysis. All plate counts were transformed to the logarithmic form (14). Means and standard deviations were calculated with GraphPad InStat version 3.01 for Windows 95 (GraphPad Software, San Diego, CA).

RESULTS

All 20 of the bovine isolates listed in Table 1 were capable colonizers of the chick cecum, able to grow to concentrations approximating or exceeding 10^8 CFU/g of cecal contents, with perhaps strain 37 an exception. Two bovine isolates, 58 and 67, were *C. coli*, and both were capable colonizers. *Campylobacter* were not recovered from the ceca of any of the unchallenged control birds. Thus, birds used in these studies were *Campylobacter*-free on arrival from the hatchery and they remained *Campylobacter*-free throughout the 2-wk duration of each trial. The ribotype of campylobacteria recovered from inoculated birds was always consistent with the ribotype of the strain used for challenge. There was no cross contamination between brooder batteries housed within the same room as *Campylobacter*-colonized birds.

DISCUSSION

Cattle often harbor *Campylobacter* (1,11,15), and cattle have been proposed as a reservoir of the organism, enabling broilers to become colonized by providing a source for the organism (5). However, metronidazole resistance patterns of *Campylobacter* isolated from both cattle and poultry indicate that there is some host-range specificity based on this phenotype and a relationship between the metronidazole resistance phenotype and the ability of *Campylobacter* strains to colonize poultry (15). In this present work, we examined the ability of strains isolated from dairy cattle in three geographic regions of the United States and found that these isolates

readily colonize the chick under laboratory conditions. Therefore, these results indicate a general ability of bovine isolates to colonize poultry. Interestingly, the two *C. coli* strains used in this present study also colonized chicks—a finding that is consistent with our previous work showing that *C. coli* isolated from swine will colonize chickens within the laboratory (21).

Different species and strains of *Campylobacter* appear to have host preferences. For example, swine are a primary natural reservoir of *C. coli*, whereas *C. jejuni* is generally found in poultry, and both species are able to infect humans. Nevertheless, human campylobacteriosis in the United States is due predominantly to *C. jejuni*. The extent to which movement of organisms between species occurs in nature or at the farm is still open. Our data reveal that cattle probably are able to serve as reservoirs and sources of *C. jejuni* colonizing poultry, both in nature and at the farm. We have shown here that bovine isolates obtained from widely different geographic locals have the ability to colonize chickens. Whether or not the organisms actually move from cattle to chickens, or the other way around, is a slightly different question not quite answered yet with the full weight of modern molecular methods.

REFERENCES

1. Aarestrup, F. M., E. M. Nielsen, M. Madsen, and J. Engberg. Antimicrobial susceptibility patterns of thermophilic Campylobacter spp. from humans, pigs, cattle, and broilers in Denmark. Antimicrob. Agents Chemother. 41:2244–2250. 1997.

2. Cox, N. A., N. J. Stern, K. L. Hiett, and M. E. Berrang. Identification of a new source of Campylobacter contamination in poultry: transmission from breeder hens to broiler chickens. Avian Dis. 46:535–541. 2002.

3. Cox, N. A., N. J. Stern, J. L. Wilson, M. T. Musgrove, R. J. Buhr, and K. L. Hiett. Isolation of Campylobacter spp. from semen samples of commercial broiler breeder roosters. Avian Dis. 46:717–720. 2002.

4. Glunder, G. Infectivity of Campylobacter jejuni and Campylobacter coli in chickens. Berl. Munch. Tieraerztl. Wochenschr. 108:101–104. 1995.

5. Gregory, E., H. Barnhart, D. W. Dreesen, N. J. Stern, and J. L. Corn. Epidemiological study of Campylobacter spp. in broilers: source, time of colonization, and prevalence. Avian Dis. 41:890–898. 1997.

6. Harvey, R. B., R. E. Droleskey, C. Sheffield, T. S. Edrington, R. O. Elder, T. R. Callaway, R. C. Anderson, D. L. J. Drinnon, and D. J. Nisbet. Campylobacter prevalence in lactating dairy cows. In: Proc. 45th Annual Conference of the American Association of Veterinary Laboratory Diagnosticians, St. Louis, MO. p. 95. 2002.

7. Hiett, K. L., N. A. Cox, and N. J. Stern. Direct polymerase chain reaction detection of Campylobacter spp. in poultry hatchery samples. Avian Dis. 46:219–223. 2002.

8. Kramer, J. M., J. A. Frost, F. J. Bolton, and D. R. Wareing. Campylobacter contamination of raw meat and poultry at retail sale: identification of multiple types and comparison with isolates from human infection. J. Food Prot. 63:1654–1659. 2000.

9. Munroe, D. L., J. F. Prescott, and J. L. Penner. Campylobacter jejuni and Campylobacter coli serotypes isolated from chickens, cattle, and pigs. J. Clin. Microbiol. 18:877–881. 1983.

10. Nielsen, E. M., J. Engberg, V. Fussing, L. Petersen, C. H. Brogren, and S. L. On. Evaluation of phenotypic and genotypic methods for subtyping Campylobacter jejuni isolates from humans, poultry, and cattle. J. Clin. Microbiol. 38:3800–3810. 2000.

11. Nielsen, E. M., J. Engberg, and M. Madsen. Distribution of serotypes of Campylobacter jejuni and C. coli from Danish patients, poultry, cattle and swine. FEMS Immunol. Med. Microbiol. 19:47–56. 1997.

12. Owen, R. J., C. Fitzgerald, K. Sutherland, and P. Borman. Flagellin gene polymorphism analysis of Campylobacter jejuni infecting man and other hosts and comparison with biotyping and somatic antigen serotyping. Epidemiol. Infect. 113:221–234. 1994.

13. Owen, R. J., and S. Leeton. Restriction fragment length polymorphism analysis of the flaA gene of Campylobacter jejuni for subtyping human, animal and poultry isolates. FEMS Microbiol. Lett. 176:345–350. 1999.

14. Snedecor, G. W., and W. G. Cochran. Statistical methods, 6th ed. Iowa State University Press, Ames, IA. 1967.

15. Stanley, K. N., and K. Jones. High frequency of metronidazole resistance among strains of Campylobacter jejuni isolated from birds. Lett. Appl. Microbiol. 27:247–250. 1998.

16. Stas, T., F. T. W. Jordan, and Z. Woldehiwet. Experimental infection of chickens with Campylobacter jejuni: strains differ in their capacity to colonize the intestine. Avian Pathol. 28:61–64. 1999.

17. Stern, N. J., D. M. Jones, I. Wesley, and D. M. Rollins. Colonization of chicks by non-culturable Campylobacter jejuni spp. Lett. Appl. Microbiol. 18:333–336. 1994.

18. Stern, N. J., M. C. Robach, N. A. Cox, and M. T. Musgrove. Effect of drinking water chlorination on Campylobacter spp. colonization of broilers. Avian Dis. 46:401–404. 2002.

19. Stern, N. J., B. Wojton, and K. Kwiatek. A differential-selective medium and dry ice generated atmosphere for recovery of Campylobacter jejuni. J. Food Prot. 55:514–517. 1992.

20. Young, C. R., R. L. Ziprin, M. E. Hume, and L. H. Stanker. Dose response and organ invasion of day-of-hatch leghorn chicks by different isolates of Campylobacter jejuni. Avian Dis. 43:763–767. 1999.

21. Ziprin, R. L., M. E. Hume, C. R. Young, and R. B. Harvey. Cecal colonization of chicks by porcine strains of Campylobacter coli. Avian Dis. 46:473–477. 2002.